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ANTHELMINTIC POTENTIAL OF CRUDE ETHANOLIC EXTRACTS OF SELECTED PLANT SPECIES

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ABSTRACT

The present article illustrates the Anthelmintic activity of crude ethanolic extracts of Azadirachta indica, Phyllanthus emblica, Carica papaya and Citrus limetta leaves. The anthelmintic assay was carried out on Indian adult earthworms (Pheretima Posthuma), as per the method of Ajaiyeoba et al., (2001) with slight modifications. The minimum time for paralysis/death of the worms, was recorded in the ethanolic extract of Citrus limetta leaves, followed by ethanolic extracts of Azadirachta indica + Carica papaya leaves (2:3 dose proportion), at 50 ppm concentration. The experimental evidences have demonstrated significant anthelmintic activity of ethanolic extracts of Azadirachta indica, Phyllanthus emblica, Carica papaya and Citrus limetta leaves, at selected optimized concentration and dose proportion combinations. Thus, selected plant species could be explored further, in terms of biochemical characterization of active molecules, coupled with dose proportion studies, that can contribute to anthelmintics with enhanced efficacy.

KEYWORDS: Anthelmintic Activity, Ethanolic Extracts, Earthworms, Azadirachta Indica, Phyllanthus Emblica, Carica Papaya & Citrus Limetta

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Original Article

INTRODUCTION

Helminthes infections, known as Helminthiasis is one of the most important animal diseases worldwide (Kosalge and Fursule, 2009; Roy *et al.*,2012; Mehta *et al.*, 2012; Dogar *et al.*, 2012; Vennila *et al.*, 2015) and probably causes more morbidity, greater economic and social deprivation, among humans and animals, than any single group of parasites, thus distressing a large proportion of world's population (Partap *et al.*, 2012). Soil transmitted Helminthiasis (STHs) affects an average of 24% people, worldwide (WHO Fact Sheets, updated 2017). Helminthiasis inflicting heavy production losses in grazing/farm animals (Dogar *et al.*, 2012; Roy *et al.*, 2012; Goswami *et al.*, 2013). Helminthiasis is more widespread in developing countries, probably due to poor sanitation, malnutrition, and poor hygiene maintenance, etc. (Wath *et al.*, 2014). Helminthiasis treatment required ideal drugs, that should offer high efficacy, broad spectrum activity, free from toxic effects and cost effectiveness (Goswami *et al.*, 2013). Anthelmintics are drugs, that either kill or expel parasitic worms (Tripathi, 2004; Pillai and Nair, 2011; Mehta *et al.*, 2012). The problems associated with the use of chemical drugs like higher cost (Vennila *et al.*, 2015), several side effects (Gogoi *et al.*, 2014; Bochala*et al.*, 2016) and development of drug resistance (Mascie-Taylor and Karin, 2003; Wath *et al.*, 2014; Vennila *et al.*, 2015) could pave the way for herbal remedies, as alternative anthelmintics (Ashok Kumar *et al.*, 2010; Yadav and Singh, 2011; Dogar *et al.*, 2012; Goswami *et al.*, 2013; Wath *et al.*, 2014; Vennila *et al.*, 2015). Herbal medicines offer ease of use (Ullah*et al.*, 2013), negligible side effects

(Mehta *et al.,* 2012; Ullah *et al.,* 2013), better potency (Mehta *et al.,* 2012) and cost effectiveness (Ullah *et al.,* 2013; Wath *et al.,* 2014), in recent years. The traditional medicines hold a great promise, as a source of effective anthelmintic, particularly in tropical developing countries, like India (Vennila *et al.,* 2015). Despite the prevalence of parasitic infections, the research on anthelmintic drug is poor (Aswar*et al.,* 2008). Therefore, in the current study, evaluation of anthelmintic activity of crude ethanolic extracts of selected plant species (*Azadirachta indica, Phyllanthus emblica, Carica papaya* and *Citrus limetta* leaves) has been reported.

RESEARCH METHODOLOGY

For In-vitro studies, Earthworms proved to be good test worms, because of their longer survival in PBS (Dogar *et al.,* 2012), easy accessibility (Vennila *et al.,* 2015), and due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Vidyarthi, 1977; Thorn *et al.,* 1977; Vigar, 1984). A/c to Goswami *et al.,* (2013), among worms, 18% of Earthworm i.e. *Pheretima Posthuma*, have been reported as targeted parasites for the evaluation of anthelmintic activity. Therefore, it was decided to carry out the In-vitro anthelmintic activity, on Indian adult earthworms (*Pheretima Posthuma*) as preliminary assay. The healthy adult earthworms were collected from the local nursery. The earthworms were maintained under normal Vermicomposting medium, with adequate supply of nourishment and water (Nisha *et al.,* 2012). The earthworms were firstly washed with normal saline, to remove all faecal matter (Mute *et al.,* 2009; Ashok Kumar *et al.,* 2010). Earthworms 6-8 cm in length (Kosalge and Fursule, 2009) and 0.2-0.3 cm in width (Nisha *et al.,* 2012; Mute *et al.,* 2009), 0.25-0.35 g in weight were used, for all experimental protocols.

Plant materials (Fresh leaves of *Azadirachta indica, Phyllanthus emblica, Carica papaya* and *Citrus limetta*) were cleansed with distilled water and allowed to dry for 15 days, under darkness. Followed to this, it was coarsely crushed using homogenizer and grinded mechanically of mesh size 1 mm. The powdered plant material was extracted with 70% Ethanol. After 48 hours, extracts were filtered by using muslin cloth (cheese cloth) followed by Whatman filter paper No.1 and filtrates were evaporated till dryness and weighed. The crude extracts were stored in air tight glass containers at 4°C till further analysis (Bibi *et al.,* 2011; Shobowale *et al.,* 2013).

Percent of the yield of Extract was calculated according to Patil and Gaikwad (2010); Ekaluo *et al.,* (2015). The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al.,* (2001) with slight modifications. Solvents used in extraction and Reagents for phytochemical and anthelmintic analysis was of pure analytical grade. All the results were expressed as a mean ± SEM of six worms in each group.

RESULTS AND DISCUSSIONS

Percentage Yield of Extract

Percent yield of the crude ethanolic extracts from *Azadirachta indica, Phyllanthus emblica, Carica papaya* and *Citrus limetta*leavesafter complete extraction were 11.91 %, 4.33 %, 8.81 % and 8.41 %, respectively. Highest yield was observed in the ethanolic extract of *Azadirachta indica* leaves followed by ethanolic extracts of *Carica papaya* and Citrus *limetta* leaves. Similar results were also reported in the previous studies. Kazeem *et al.,* (2013) found, extract yield of 4.35 % and 2.25 % in aqueous and ethanolic extracts of *Azadirachta indica* leaves, respectively. A/c to Sithisarn *et al.,* (2006), Maceration, Percolation and Soxhlet extraction of *Azadirachta indica* leaves with concentrations of ethanol ranges from 20-95%, gave a crude extract yield b/w 10-25% w/w. Chavez-Quintal *et al.,* (2011) in his studies, observed the yield of ethanolic extracts of *Carica papaya* leaves in the range of 3.1-5.9 % under variable extraction time and flour: solvent ratio.

Gitika and Kumar (2016) were reported % extract yield of 10.52 and 15.41 in ethanolic and aqueous extracts of *Phyllanthus emblica* leaves, respectively. 19.25 % of extract yield was observed by Muthiah *et al.,* (2012) in 60% ethanolic extract of *Citrus limetta* leaves and peels.

Anthelmintic Activity

In order to investigate the anthelmintic properties of *Azadirachta indica, Phyllanthus emblica, Carica papaya and Citrus limetta*leaves, the present study has been conducted with ethanolic extracts optimized at different concentrations and dose proportion combinations. Albandazole (at 10, 15 & 20 mg/ml) served as standard throughout the experiment. Ethanolic extracts of *Azadirachta indica*, *Phyllanthus emblica, Carica papaya* and Citrus *limetta* leaves produced a significant anthelmintic activity in a dose dependent manner. It was observed that the ethanolic extract of *Carica papaya* leaves has a more profound effect as anthelmintic, than the ethanolic extract of *Azadirachta indica* leaves at 50 ppm concentration. The higher anthelmintic potential of mixed dilutions of *Azadirachta indica* and *Carica papaya* at the dose proportion of 2:3 over 3:2 dose proportion at 50 ppm concentration also justified the same. However, minimum time for paralysis/death of the worms were recorded in the ethanolic extract of *Citrus limetta* leaves, as well as in mixed dilutions of *Azadirachta indica* and *Carica papaya* (2:3 dose proportion), at 50 ppm concentration.

In earlier studies, Aggarwal and Bagai (2014), confers that, ethanolic and aqueous extracts of *A. indica* leaf may act as potential Vermifuge or Vermicide. Roy *et al.,* (2012) reported the significant anthelmintic property of hydro-alcoholic and chloroform extracts of the stem and leaves of *Carica papaya* at 5%, 2.5% and 1% extract concentrations, against *Pheretima Posthuma.* Sukanya *et. al.,* (2013) studied anthelmintic activity of methanol, butanol, ethyl acetate and aqueous extracts of *Phyllanthus emblica* leaves & bark, against *Pheretima Posthuma.* Barrage *et al.,* (2011), demonstrated anthelmintic activity of petroleum ether extract of *Citrus medica* (L.) leaves, against *Pheretima Posthuma.*

CONCLUSIONS

The experimental evidences have demonstrated significant anthelmintic activity of ethanolic extracts of *Azadirachta indica, Phyllanthus emblica, Carica papaya* and *Citrus limetta* leaves, as they took less time to cause paralysis and death of the earthworms, at selected optimized concentration and dose proportion combinations. The present findings were justified from the comparative analysis with standard drug Albandazole, at different doses. However, the findings of present study did not exclude the possibility that, the ethanolic extracts of studied plant species under variable concentrations and dose proportion combinations, having lower anthelmintic activity, might be efficacious against other species of helminths. It is recommended that, the selected plant species could be explored further, in terms of biochemical characterization of active molecules, coupled with dose proportion studies that can contribute to anthelmintics with enhanced efficacy.

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